Under Siege: The Brain on Opiates

Minireview

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Many of the terms used in the addiction field today were developed originally based on the opiates, one of the oldest and best characterized classes of abused drugs. Tolerance describes the need for an increasing dose of opiate to achieve the same effect; this term was based originally on the tolerance that develops to many of the acute behavioral actions of opiates (e.g., analgesia, autonomic inhibition, and "high"). Dependence describes an altered physiological state caused by repeated opiate exposure such that cessation of drug administration leads to a withdrawal syndrome characterized by serious physiological disturbances. This term was used originally to describe physical opiate dependence as characterized by classical opiate withdrawal (i.e., "cold turkey"). More recently, it has become clear that opiates also result in a psychological form of dependence, which results in emotional or motivational symptoms upon drug withdrawal. This latter form of dependence is probably the strongest determinant of opiate addiction, which can be defined as compulsive drug craving and administration despite horrendous adverse consequences.

The classical distinction between physical and psychological forms of dependence maps onto partially distinct regions of the CNS that underlie these phenomena (Koob, 1992). The analgesic and autonomic effects of opiates, and the bulk of physical withdrawal symptoms, have been ascribed largely to drug action in the brain stem and spinal cord. In contrast, the acute rewarding effects of opiates, and the motivational symptoms of withdrawal, have been ascribed largely to the mesolimbic dopamine system and other limbic structures (e.g., amygdala and hippocampus; Koob, 1992; Wise, 1990). Identification of specific neural circuits responsible for various actions of opiates has made it possible to begin the process of identifying the relevant molecular and cellular events that underlie the altered functioning of these circuits.

Cellular and Molecular Adaptations Underlying Opiate Tolerance and Dependence

The discovery of endogenous opioid peptides and opioid receptors over twenty years ago led to the expectation that adaptations in these proteins in response to repeated opiate exposure underlie many features of opiate addiction. While investigation of the opioid peptides enkephalin, endorphin, and dynorphin has revealed a great deal about peptide neurotransmitters in general and a role for the opioid peptides in stress-related phenomena (Olsen et al., 1994), adaptations in the expression of peptides themselves have not provided compelling models for opiate tolerance or dependence. Similarly, early studies of opiate regulation of the three types of opioid receptor, μ, δ, and κ, by use of radioligand binding assays failed to explain opiate tolerance and dependence, at least in in vivo systems. The recent cloning of these receptors has enabled more penetrating analyses (Mansour et al., 1995), which could yet reveal alterations related to opiate addiction. For example, exposure to agonist in vitro elicits receptor desensitization and down-regulation, which could be related to aspects of tolerance in vivo (e.g., Harris and Williams, 1991; Von Zastrow et al., 1994).

In contrast with the lack of evidence for a role of adaptations in opioid peptides and receptors in opiate addiction, considerable evidence has implicated a role for postreceptor, intracellular messenger pathways. Chronic exposure to opiates has been shown to elicit adaptations in some of the same intracellular pathways that mediate the acute actions of the drugs. Moreover, such adaptations have been related to tolerance and dependence phenomena that can be demonstrated at the level of individual neurons.

Studies of the Locus Coeruleus

The locus coeruleus (LC), the major noradrenergic nucleus in brain, is located in the brain stem and involved in control of autonomic function and attentional states. Activation of this brain region, and the resulting increase in noradrenergic innervation of large regions of the brain and spinal cord, is one of the major determinants of physical opiate withdrawal (Koob et al., 1992; Nestler et al., 1993, 1994). Since LC neurons are extremely well characterized electrophysiologically, and are clustered in a densely packed, relatively homogeneous nucleus, they have served as a useful model of opiate action.

Acutely, opiates, acting at μ receptors, inhibit LC neurons in part by activating an inward rectifying K^+ current through coupling with the $G_{i/o}$ family of G proteins (Figure 1). Opiate inhibition is also achieved by inhibiting an inward nonspecific cation current, an effect mediated via inhibition of adenylyl cyclase and reduced activity of protein kinase A (PKA) (Alreja and Aghajanian, 1993). The channel or pump responsible for this current has not yet been identified, so it remains to be established whether PKA phosphorylates the pump or channel directly. LC neurons develop cellular forms of tolerance and dependence; in the continued presence of opiates, LC neurons gradually recover from the acute inhibitory effect of the drug, whereas upon removal of the drug, LC neurons show a manyfold activation above control levels (for review, see Nestler et al., 1994).

We now know that these changes are mediated in part by an up-regulation of the cAMP pathway induced by chronic exposure to opiates. Thus, chronic opiate administration increases levels of expression of adenylyl cyclase and PKA in the LC (Nestler et al., 1993, 1994; Matsuoka et al., 1994), adaptations that increase the intrinsic excitability of the neurons via activation of the nonspecific cation current. In the chronic-treated state, the combined presence of the up-regulated cAMP pathway and of the opiate helps return LC neurons to their normal firing rates. Upon removal of the opiate, the up-regulated cAMP pathway is unopposed and contributes to the activation of LC neurons during withdrawal.

Additional mechanisms are likely to be involved in

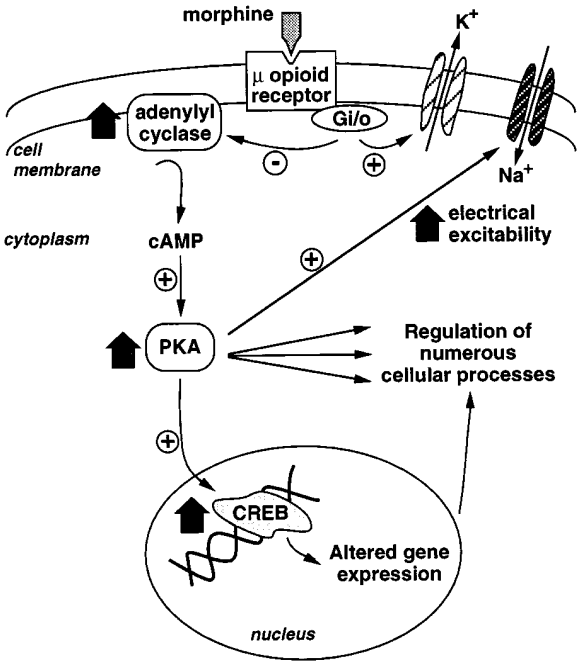

Figure 1. Scheme Illustrating Opiate Actions in the LC

Opiates acutely inhibit LC neurons by increasing the conductance of a K^+ channel (light cross-hatch) via coupling with subtypes of G_i and/or G_o and by decreasing a Na^+-dependent inward current (dark cross-hatch) via coupling with $G_{i/o}$ and the consequent inhibition of adenylyl cyclase. Reduced levels of cAMP decrease PKA activity and the phosphorylation of the responsible channel or pump. Inhibition of the cAMP pathway also decreases phosphorylation of numerous other proteins and thereby affects many additional processes in the neuron. For example, it reduces the phosphorylation state of CREB, which may initiate some of the longer-term changes in LC function. Upward bold arrows summarize the effects of chronic morphine in the LC. Chronic morphine increases levels of adenylyl cyclase, PKA, and several phosphoproteins, including CREB. These changes contribute to the altered phenotype of the drug-addicted state. For example, the intrinsic excitability of LC neurons is increased via enhanced activity of the cAMP pathway and Na^+-dependent inward current, which contributes to the tolerance, dependence, and withdrawal exhibited by these neurons. This altered phenotypic state may be maintained in part by up-regulation of CREB expression.

tolerance in LC neurons, since up-regulation of the cAMP pathway would not appear to explain the diminished ability of opiate agonists to activate K^+ channels, which occurs independently of this pathway. Several possibilities exist but have yet to be directly implicated in the LC or another in vivo system. Opioid receptors may be functionally uncoupled from their G proteins, perhaps through phosphorylation of the receptors or G protein subunits. For example, opioid receptors can be desensitized upon phosphorylation by G protein receptor kinases (e.g., βARK; Pei et al., 1995), and such kinases are up-regulated in the LC following chronic morphine treatment (Nestler et al., 1994). Opiate-induced down-regulation of $G_{i/o}\alpha$ subunits, which has been observed in several neuronal cell types, could also be involved. Although $G_{i/o}\alpha$ immunoreactivity in the LC is increased by chronic morphine exposure, the functioning of the proteins, based on measures of GTPase activity, is reduced (Sim et al., 1996). Finally, regulation of the K^+ channels themselves has been implicated (Kovoor et al., 1995).

The mechanism by which chronic opiate exposure leads to up-regulation of the cAMP pathway in LC neurons is the focus of ongoing investigation. One model, depicted in Figure 1, proposes a role for the transcription factor cAMP response element–binding protein (CREB). Levels of CREB are increased in the LC following chronic morphine administration (Widnell et al., 1994). This raises the possibility that increases in CREB contribute to up-regulation of the other cAMP pathway proteins and thereby to the effects of chronic opiates on the electrophysiological state of LC neurons and their behavioral manifestations. The current challenge is to study this possibility directly. For example, intra-LC administration of antisense oligonucleotides to CREB, or genetic mutant mice deficient in CREB, would be expected to show diminished effects of chronic opiates in the LC, whereas viral-mediated increases in CREB expression in this brain region would be expected to mimic some of the effects of chronic opiates. Work along these lines is underway in several laboratories.

Activation of the LC during opiate withdrawal also depends upon factors extrinsic to the LC, namely an increase in glutamatergic neurotransmission to the LC from the medullary nucleus paragigantocellularis (Akaoka et al., 1991; Nestler et al., 1994). The factors that drive this activation of the paragigantocellularis remain unknown, but appear to involve several medullary and spinal nuclei. Up-regulation of the cAMP pathway, which occurs in some of these regions, may be one of the mechanisms responsible for activation of these proximal neural circuits. However, it should be emphasized that up-regulation of the cAMP pathway is probably one of many molecular adaptations responsible for opiate tolerance and dependence at the cellular level (Childers, 1991).

Motivational Dependence and the Mesolimbic Dopamine System

As mentioned above, the mesolimbic dopamine system plays an important role in acute opiate reinforcement and the changes in reinforcement mechanisms that characterize addiction. This neural system consists of dopaminergic neurons in the ventral tegmental area (VTA) and the limbic brain regions to which they project, such as the nucleus accumbens (NAc) and prefrontal cortex.

The mesolimbic dopamine system is thought to regulate motivational behavior to natural reinforcers such as food and sex. The changes that chronic opiate exposure elicits in the VTA and NAc that are responsible for motivational dependence remain poorly understood. Acutely, opiates increase dopaminergic signals to the NAc via activation of VTA dopamine neurons. This activation occurs indirectly through inhibition of inhibitory GABAergic interneurons in the VTA (Johnson and North, 1992). Opiates also directly affect NAc neurons independently of dopamine via activation of opioid receptors expressed by these neurons. The relative contribution of these two sites of opiate action to the acute reinforcing

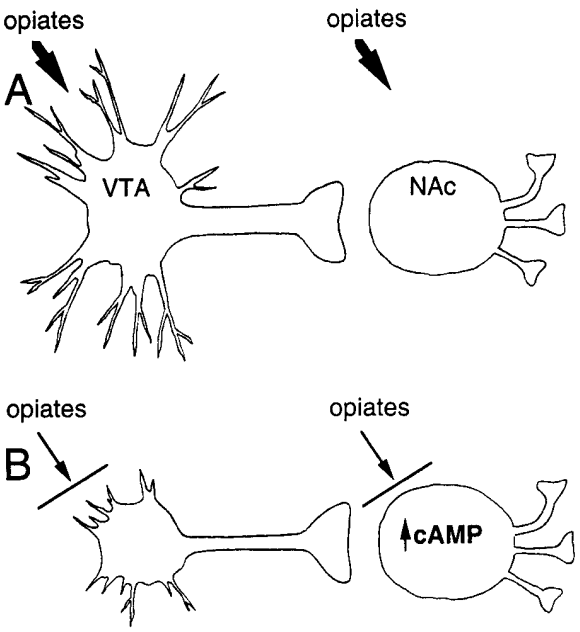

Figure 2. Scheme Illustrating Changes in Mesolimbic Dopamine Function Associated with Opiate Addiction

(A) VTA neuron projecting to an NAc neuron under normal conditions. (B) The same neurons after chronic opiate exposure. Acutely, opiates produce potent effects both in the VTA and in the NAc. After chronic exposure, opiates produce long-term adaptations in both regions, which oppose these acute effects. In the VTA, there is a gross reduction in the size and, presumably, functioning of these neurons, which would oppose their activation by acute opiate exposure. In the NAc, there is an up-regulation of the cAMP pathway, which would oppose acute opiate inhibition of this pathway. Upon removal of the opiate, these adaptations would lead to impairment in mesolimbic dopamine function, which could contribute to an aversive state during withdrawal.

effects of opiates remains unknown (Wise, 1990; Koob, 1992). Nevertheless, the intensity and duration of these acute actions of opiates, which would mediate a drug "high," far exceed the activation of the VTA–NAc that occurs under normal conditions. This opioid-mediated activation could elicit compensatory adaptations in VTA and NAc neurons to counter or impair the acute reactivity of these systems (Figure 2). Such adaptations could underlie motivational dependence by causing deficient VTA–NAc function and thereby an aversive state during periods of drug withdrawal, changes alleviated by further drug exposure.

Despite considerable effort, it has not been possible to fully account for alterations in VTA–NAc functioning, or the related behavioral phenomena, at the level of neurotransmitters and receptors (Koob, 1992; Self and Nestler, 1995). There is some consensus that levels of extracellular dopamine in the NAc, as measured by in vivo microdialysis, may be diminished during early phases of opiate withdrawal and potentiated during later phases, but little is known about the molecular and cellular adaptations that might underlie such phenomena. Similarly, consistent changes in opioid or dopamine receptors following long-term opiate exposure have not been observed. In contrast, as with studies of opiate action elsewhere, adaptations in intracellular signaling pathways have been illuminating.

Up-Regulation of the cAMP Pathway in the NAc

Chronic morphine treatment increases levels of adenylyl cyclase and PKA in the NAc, as observed in the LC (Nestler et al., 1993; Self and Nestler, 1995). There is also a reduction in levels of $G_{i/o}\alpha$ subunits, which would further increase cAMP function. Similar effects are seen upon chronic self-administration of heroin or chronic exposure to other drugs of abuse. Up-regulation of the cAMP pathway has been related directly to the acute reinforcing effects of opiates. Direct administration of agents into the NAc that inhibit $G_{i/o}$ or activate protein kinase reduces the acute reinforcing effects of opiates (Self et al., 1994; Self and Nestler, 1995). Agents that produce the opposite effect potentiate opiate reinforcement. These findings are consistent with the view that up-regulation of the cAMP pathway that occurs in response to chronic opiate exposure could be one mechanism of tolerance and dependence to the motivational effects of opiates (Figure 2); this adaptation would oppose acute opiate reinforcement as well as lead to an aversive motivational state during opiate withdrawal.

One of the challenges in delineating the functional consequences of molecular adaptations in the NAc is the complexity of this brain region and its afferent and efferent connections. Unlike the LC, the NAc contains several types of neurons that may well play very different roles in drug reinforcement mechanisms. Also, unlike the LC, it is less clear how these NAc cells are influenced by their many inputs (e.g., VTA, prefrontal cortex, and amygdala) and how the outputs of the cells modify neural circuits to regulate drug reinforcement and related behaviors. Future studies are needed to identify the specific cell types in the NAc wherein up-regulation of the cAMP pathway and other adaptations occur and how these adaptations alter the functioning of these neurons as well as the neural circuits in which they operate.

Adaptations in the VTA

The changes elicited by chronic opiate exposure in the VTA are more complex. Chronic drug treatment induces higher levels of tyrosine hydroxylase (the rate-limiting enzyme in the biosynthesis of dopamine) and of specific glutamate receptor subunits in this region (Nestler et al., 1993; Fitzgerald et al., 1996). Moreover, there appear to be major structural adaptations, perhaps even neural injury, within this brain region. Chronic opiate exposure, including self-administered heroin, decreases levels of neurofilament proteins and increases levels of glial fibrillary acidic protein specifically in this brain region. As would be expected, the reduction in neurofilament proteins is associated with impaired axoplasmic transport from the VTA to the NAc as well as with a reduction in the mean size of individual VTA dopamine neurons (Self and Nestler, 1995; Sklair-Tavron et al., 1995, Soc. Neurosci., abstract). Studies of the chronic actions of opiates on the physiological activity of the VTA are needed to complement these findings. Nevertheless, the observed adaptations are consistent with a gross impairment of VTA dopamine neurons, which, like up-regulation of the cAMP pathway in the NAc, can be viewed as a compensatory response to acute opiate activation of the cells (Figure 2). Such impairment in VTA function

could then contribute to motivational dependence and an aversive state during opiate withdrawal.

Neurotrophic Factors and Opiate Dependence

The nature of opiate-induced neural plasticity observed in the VTA suggests a potential role for neurotrophic factors in these phenomena. Neurotrophic factors, although studied originally for their role in neural growth and development, are now known to regulate signal transduction and neuronal viability in the fully differentiated adult brain. Indeed, recent studies have demonstrated that certain neurotrophic factors can pharmacologically modify opiate action in the mesolimbic dopamine system (Berhow et al., 1995). Administration of brain-derived neurotrophic factor (BDNF) or related neurotrophins, directly into the VTA, both prevents and reverses some of the effects that opiates elicit in this brain region (e.g., induction of tyrosine hydroxylase and glial fibrillary acidic protein and structural changes in VTA dopamine neurons). Intra-VTA infusions of BDNF also attenuate up-regulation of the cAMP pathway observed in the NAc.

More recent investigations have provided direct evidence that perturbation of neurotrophin signaling pathways may also play a role in mediating some of the effects of opiates on the VTA (Berhow et al., 1995, Soc. Neurosci., abstract). Chronic morphine administration results in a sustained activation of extracellular signal-regulated kinases (ERKs), the major effector for BDNF and related neurotrophins, specifically in the VTA. This activation appears to mediate the effect of chronic opiate exposure on certain other adaptations in this brain region, such as induction of tyrosine hydroxylase. While the precise role of neurotrophic factors in opiate addiction remains to be established, this work illustrates the array of cellular mechanisms that must be considered in understanding drug-induced adaptations in neural functioning.

Glutamatergic Neurotransmission and Opiate Tolerance and Dependence

There have been numerous reports that chronic coadministration of glutamate receptor antagonists, particularly N-methyl-D-aspartic acid (NMDA) antagonists, can prevent the development of tolerance and dependence in response to chronic opiate administration (Trujillo and Akil, 1995). There also is growing evidence for regulation of the VTA–NAc pathway by glutamatergic inputs from cerebral cortex and elsewhere (Kalivas, 1995). However, the interactions between NMDA receptor antagonists and opiates are more complex than first appreciated. Like opiates, NMDA antagonists (including phencyclidine [PCP] and MK-801) have powerful stimulant and reinforcing actions of their own and can potentiate the activating and reinforcing effects of opiates (Carlezon and Wise, 1993). These findings suggest that chronic coadministration of NMDA receptor antagonists may make opiates more addictive regardless of their effects on tolerance and physical dependence. Clearly, more work is needed to characterize the molecular and cellular basis of the complex interactions between these classes of addictive drugs.

Conclusions

Opiate-induced adaptations in neuronal function involve molecular adaptations in components of signaling pathways that go far beyond regulation of endogenous opioid peptides and opioid receptors or other neurotransmitter receptor systems. Increasing evidence supports a role for adaptations in the cAMP pathway as one important molecular mechanism of opiate tolerance and dependence. Adaptations in many other signaling pathways are also likely to be involved. One example is opiate regulation of the neurotrophin–ERK signaling cascade and its possible role in mediating some of the long-term effects of opiates on the brain. Opiate-induced changes in the structural features of VTA dopamine neurons further highlight the complex types of adaptations that are elicited in the brain by chronic drug exposures. Indeed, these structural changes lend support to growing evidence for neural plasticity in the brain that is detectable at the anatomical level. The availability of accurate animal models of opiate addiction enable the exploration of these various mechanisms of neural plasticity within functional and clinically relevant contexts.

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